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GBV-C/HGV Infection in Children With Chronic Hepatitis C

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The role of GB virus-C/hepatitis G virus (GBV-C/HGV), a recently identified member of the *Flaviviridae* family, in children with liver disease is not well understood. The aims of this study were to evaluate the prevalence of GBV-C/HGV and to clarify its pathogenic role in young patients with chronic hepatitis C. Sixty-four Japanese children and adolescents with chronic hepatitis C virus (HCV) infection, with a mean age of 9.8 years, were evaluated retrospectively. Twenty-one (32.8%) of the 64 patients were positive for serum GBV-C/HGV RNA. Only 1 (1.6%) of the 64 patients was positive for antibody against the envelope protein E2 of GBV-C/HGV (anti-E2) and GBV-C/HGV. None of them was positive for anti-E2 alone. There was no significant difference in clinical, virological, or histological characteristics between GBV-C/HGV-positive and GBV-C/HGV-negative patients, except for underlying malignant disease. There was no evidence that GBV-C/HGV might affect the response of HCV to interferon therapy in young patients with chronic hepatitis C. The prevalence of GBV-C/HGV infection in young patients with chronic hepatitis C is similar to that in adult patients with chronic hepatitis C, but E2-seroconversion is observed infrequently. Underlying malignant disease is a risk factor for GBV-C/HGV viremia. GBV-C/HGV does not seem to affect the clinical course of young patients with chronic hepatitis C. ***J. Med. Virol. 59:154–159, 1999.***

KEY WORDS: GBV-C; HGV; HCV; anti-E2; children; interferon

Recently, a novel RNA virus designated as GB virus type C (GBV-C)/hepatitis G virus (HGV), belonging to the *Flaviviridae* family, has been identified in patients with non-A-E hepatitis by two independent groups [Simons et al., 1995; Linnen et al., 1996]. This virus is transmissible by blood transfusions, blood products, intravenous drug use, and hemodialysis [Aikawa et al., 1996; Linnen et al., 1996; Masuko et al., 1996; Schmidt et al., 1996]. Previous studies have revealed that the prevalence of GBV-C/HGV infection is in the range of 1–2% among volunteer blood donors in the USA and Europe and 15–20% among intravenous drug users as determined by the reverse transcription-polymerase chain reaction (RT-PCR) method [Jarvis et al., 1996; Linnen et al., 1996; Alter et al., 1997]. Initially, several studies reported that GBV-C/HGV is associated with acute, chronic non-A-E hepatitis, fulminant hepatitis, and aplastic anemia [Yoshiba et al., 1995; Byrnes et al., 1996; Alter et al., 1997]. Recently, its clinical significance in cases of human hepatitis in adults has become increasingly controversial.

Although some studies have been conducted in children [Bortolotti et al., 1997; Chen et al., 1997; Chung et al., 1997; Lai et al, 1997; Lopez-Alcorocho et al., 1997; Mison et al., 1997; Szabo et al., 1998], little is known about the virological, serological, biochemical, and histological characteristics of GBV-C/HGV infection in children. In this retrospective study, we investigated the clinical, virological, and histological implications of a concomitant GBV-C/HGV infection in children with hepatitis C virus (HCV)-related liver disease.

PATIENTS AND METHODS

Patients

Sixty-four Japanese children and adolescents with chronic HCV infection (male/female ratio = 45/19; mean age = 9.8 ± 4.6 years; range, 6 months to 19 years) were studied. Thirty-six of the 64 patients had a history of blood transfusion and an underlying malignant disease: acute lymphoblastic leukemia in 15 patients, acute myelogenous leukemia in 10 patients, aplastic anemia in 4 patients, neuroblastoma in 2 patients, chronic myelogenous leukemia, malignant lymphoma, hepatoblastoma, rhabdomyosarcoma, and viral associated hemophagocytic syndrome each in 1 patient.

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Seventeen patients had a history of blood transfusion or use of blood products, but they did not have any underlying malignant disease. Six patients were born to mothers known to be HCV carriers at delivery. In the remaining 5 patients, the possible source of HCV infection could not be identified. The diagnosis of chronic hepatitis was based on a documented elevation of alanine aminotransferase (ALT) levels for at least 6 months, the presence of anti-HCV antibody, and histological findings on liver biopsies. All patients were negative for hepatitis B surface antigen and for antibody to hepatitis B core antigen, and negative for IgM antibodies against cytomegalovirus and Epstein-Barr virus. Other causes of liver damage such as α1-antitrypsin deficiency, hemochromatosis, and Wilson's disease were also ruled out. Anti-HCV antibody was detected using a second-generation enzyme immunoassay (Abbott HCV EIA 2.0, Tokyo, Japan). Serum HCV RNA was detected by nested RT-PCR with two pairs of inner and outer (nested) primers with sequences corresponding to the 5′ untranslated region of HCV [Okamoto et al., 1990; Inui et al, 1995]. HCV genotyping was carried out before the initiation of interferon (IFN) therapy by nested RT-PCR using type-specific primers with sequences corresponding to the HCV core region [Okamoto et al., 1992]. Serum HCV RNA levels were quantified using a multicyclic RT-PCR method [Ishiyama et al., 1993]. Sera obtained from patients before IFN therapy were used for detection of GBV-C/HGV RNA and anti-E2 antibody.

Liver biopsies were obtained from 43 patients before the initiation of IFN therapy, and a histological diagnosis was made according to the histology activity index scoring system of Knodell et al. [1981]. Biopsy specimens were examined by an independent pathologist not connected with this study.

RNA Extraction From Serum and Detection of GBV-C/HGV RNA

Serum GBV-C/HGV RNA was detected by RT-PCR using two primer pairs with sequences corresponding to the highly conserved 5′ untranslated region of the viral genome. GBV-C/HGV RNA extracted from 200 μl of serum by the proteinase K/phenol-chloroform method was reverse-transcribed with 20 pmol of each outer primer, 10 units of reverse transcriptase (ALV Reverse Transcriptase; Life Science, St. Petersberg, FL), and 2 units of Tth DNA polymerase (Toyobo, Osaka, Japan) in a reaction mixture with a final volume of 100 μl. The reaction mixture was incubated at 37°C for 1 hr for reverse transcription. The cDNA was then amplified by nested PCR with 20 pmol of each oligonucleotide primer (outer sense: 5′-GGTTGGTAGGTCGTAAATCCCGGTCA-3′ [nt 131–156], outer antisense: GACATTGAAGGGCGACGTGGACCGTAC-3′ [nt 327–353], inner sense: TGGTAGCCACTATAGGTGGGT-3′ [nt 160–180], inner antisense: 5′-GCGACGTGGACCGTACGTGGGCGT-3′ [nt 319–342]) reported by Orito et al. [1996]. Nucleotide positions are numbered as for PNF2161 (GenBank/EMBL accession number U44402). The first round of PCR was started immediately after reverse transcription and performed for 30 cycles, consisting of denaturation at 94°C for 1 min, annealing at 50°C for 45 sec, and extension at 72°C for 1 min. Twenty microliters of PCR reaction mixture containing 20 pmol of each inner primer and 2 U Tth DNA polymerase were added to the first round PCR product. The second round of PCR was performed for 35 cycles as follows: denaturation at 94°C for 1 min, annealing at 55°C for 45 sec, and extension at 72°C for 1 min. All nested PCR reactions were performed in a thermal cycler. Products of the expected size (183 bp) were visualized under ultraviolet light on 2% agarose gels after ethidium bromide staining. One sample of negative control serum, extracted in parallel, was always included in this protocol.

Enzyme-Linked Immunosorbent Assay for GBV-C/HGV E2 Antibody

Anti-E2 was detected by means of a commercially available two-step enzyme-linked immunosorbent assay (Anti-HGenv; Boehringer Mannheim, Penzberg, Germany). Results were interpreted according to the manufacturer's instructions. In brief, the cut-off value was calculated using kit-specific positive and negative controls by means of the formula: cut-off value = 0.2× positive control + negative control. Samples were considered positive for anti-E2 at a sample/cut-off ratio of more than 1.15. Samples with a sample/cut-off ratio in the range of 0.85 and 1.15 were defined as showing borderline reactivity. All positive and borderline samples were retested in parallel by the confirmation procedure.

IFN Therapy

Twenty-nine of the 64 patients received IFN treatment for chronic hepatitis C. Informed consent was obtained from all of the patients' parents before the initiation of IFN therapy. IFN therapy was initiated at least 2 years after the completion of treatment for any underlying malignant disease. Natural IFN-alpha (Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan or Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was administered at a dose of 0.1 MU per kilogram (maximum dose: 8.0 MU) daily for 2 weeks, and then three times a week for an additional 22 weeks (total dose; 8.0 MU/kg). The response to IFN therapy was classified as a "complete response (CR)" if ALT values/HCV RNA returned to normal within 6 months after the cessation of therapy and remained normal for at least 6 months thereafter. "No response (NR)" was simply defined as any condition other than CR.

Statistical Analysis

Results are presented as the mean ± SD. Frequency distributions were compared using the chi-squared test or Fisher's exact test, and means were compared using Student's *t*-test or the Mann–Whitney test. A *P*-value of .05 or less was considered to indicate statistical significance.

TABLE I. Clinical and Virological Characteristics of Patients With and Without GBV-C/HGV RNA in Association With Chronic HCV Infection

Characteristic	GBV-C/HGV RNA		P
	Positive (n = 21)	Negative (n = 43)	
Gender (male/female)	16 (76.2%)/5 (23.8%)	30 (69.8%)/13 (30.2%)	.81
Age (years, mean ± SD)	10.0 ± 4.5	9.6 ± 4.6	.74
Underlying disease			<.05
Malignancy	16 (76.2%)	20 (46.5%)	
No malignancy	5 (23.8%)	23 (53.5%)	
Serum ALT levels (IU/L)	134.5 ± 195.0	92.3 ± 92.5	.36
Serum HCV RNA levels (copies/mL)	$10^{7.2\pm1.9}$ (n = 13)	$10^{6.7\pm1.8}$ (n = 31)	.41
HCV genotype (n = 42)			.57
1b[a]	10 (71.4%)	16 (57.1%)	
Other type	4 (28.6%)	12 (42.9%)	
Histology activity index score	6.1 ± 2.8 (n = 15)	6.0 ± 3.9 (n = 28)	.93
Anti-E2 positive	1 (4.8%)	0	.73

GBV-C, GB virus type C; HGV, hepatitis G virus; HCV, hepatitis C virus; ALT, alanine aminotransferase.
[a]Including co-infection with other genotype.

TABLE II. Response to IFN Therapy

Characteristic	GBV-C/HGV RNA		P
	Positive (n = 6)	Negative (n = 23)	
Gender (male/female)	5 (83.3%)/1 (16.7%)	16 (69.6%)/7 (30.4%)	.87
Age (years, mean ± SD)	9.8 ± 4.0	10.8 ± 4.0	.60
Underlying disease			.83
Malignancy	4 (66.7%)	14 (60.9%)	
No malignancy	2 (33.3%)	9 (39.1%)	
Serum ALT levels (IU/L)	42.0 ± 39.0	91.3 ± 97.6	.06
Serum HCV RNA levels (copies/mL)	$10^{7.5\pm1.1}$	$10^{6.8\pm2.0}$	.42
HCV genotype (n = 27)			.95
1b[a]	5 (83.3%)	15 (71.4%)	
Other type	1 (16.7%)	6 (28.6%)	
Histology activity index score	6.5 ± 2.9	6.1 ± 3.9	.81
Response to IFN therapy			.99
Complete response	2 (33.3%)	10 (43.5%)	
No response	4 (66.7%)	13 (56.5%)	

IFN, interferon; GBV-C, GB virus type C; HGV, hepatitis G virus; ALT, alanine aminotransferase; HCV, hepatitis C virus.
[a]Including co-infection with other genotype.

RESULTS

Detection of GBV-C/HGV RNA and Anti-E2 Antibody

Twenty-one (32.8%) of the 64 patients were positive for GBV-C/HGV RNA. All 21 patients had a history of blood transfusion or use of blood products. Of the 21 patients, 16 (76.2%) patients had an underlying malignant disease. Only 1 (1.6%) of the 64 patients, a 17-year-old girl with an underlying malignant disease, was positive for anti-E2. This patient was also positive for GBV-C/HGV RNA.

Characteristics of the Patients With and Without GBV-C/HGV RNA

Clinical, virological, and histological characteristics in relation to GBV-C/HGV RNA are shown in Table I. No significant differences were observed with respect to gender, age, serum ALT levels, serum HCV RNA levels, HCV genotype, histology activity index score, or anti-E2 between GBV-C/HGV RNA-positive and -negative patients. However, there was a significant difference in underlying malignant disease between the two groups.

Influence of GBV-C/HGV Infection on the Efficacy of IFN Therapy for HCV

Six (20.7%) of the 29 patients who received IFN therapy were positive for GBV-C/HGV RNA. Two (33.3%) of the 6 patients responded completely. There was no significant difference in efficacy of IFN therapy for HCV infection between GBV-C/HGV RNA-positive patients and GBV-C/HGV RNA-negative patients (Table II).

Response of GBV-C/HGV RNA to IFN Therapy

Serial samples of sera obtained from the 6 patients who were positive for GBV-C/HGV RNA (male/female ratio = 5/1; mean age = 9.8 ± 4.0 years; age range, 4–13 years) and who received IFN therapy were examined by RT-PCR. Figure 1 shows the changes in HCV RNA and GBV-C/HGV RNA. Cases 1, 3, and 4 were included in our previous studies [Fujisawa et al., 1995; Komatsu et al., 1996]. GBV-C/HGV RNA disappeared 3

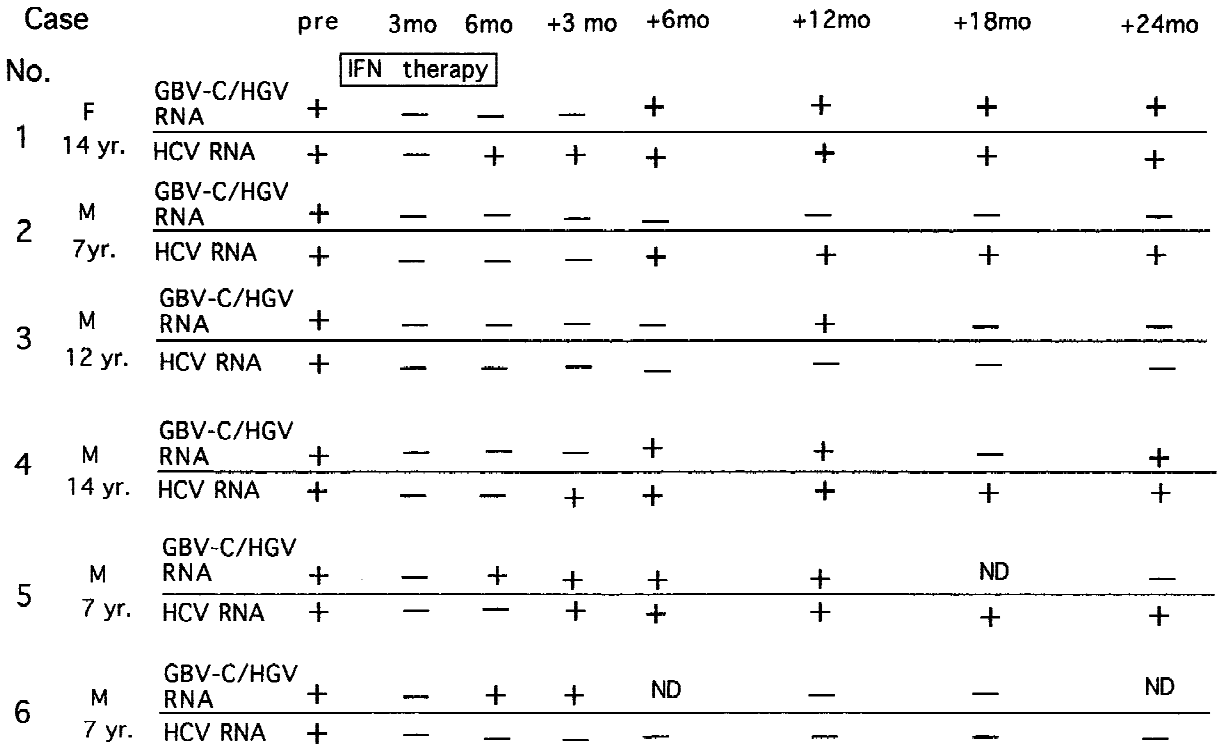

Fig. 1. Changes in serum hepatitis G virus (HGV) RNA and hepatitis C virus (HCV) RNA in six patients before, during and after interferon (IFN) therapy. Plus signs indicate the presence of HGV RNA or HCV RNA. Minus signs indicate the absence of detectable HGV RNA and HCV RNA. Cases 1, 3, and 4 were included in our previous studies. ND, not done.

months after the initiation of IFN therapy in all cases. However, 5 (83.3%) of the 6 patients became positive for GBV-C/HGV RNA again at or after the completion of IFN therapy. Only 1 patient (case 2), who showed no response to IFN therapy, was persistently negative for GBV-C/HGV RNA.

DISCUSSION

Previous studies have shown that GBV-C/HGV infection is frequent in adult patients with HCV infection [Enomoto et al., 1998; Oshita et al., 1998; Petrik et al., 1998; Shev et al., 1998]. In those studies, 10–25% and 30–50% of adult patients with chronic hepatitis C were positive for GBV-C/HGV RNA and anti-E2, respectively. As the presence of anti-E2 is thought to indicate resolved infection [Tacke et al., 1997; Enomoto et al., 1998; Shev et al., 1998], the overall prevalence of GBV-C/HGV exposure (either positive for GBV-C/HGV RNA or anti-E2) may be approximately half of all adult patients with chronic hepatitis C. However, the overall prevalence of GBV-C/HGV exposure in children with chronic hepatitis C had not been examined previously.

In our study, the prevalence of GBV-C/HGV viremia was 32.8% among young patients with chronic hepatitis C and it was higher than that for HCV-infected adult patients. However, anti-E2 was present in only one (1.6%) patient and the overall prevalence of GBV-C/HGV exposure was similar to or slightly lower than that found for adult patients. These findings seem to support two hypotheses. First, the ability to eliminate GBV-C/HGV is influenced by the age of patients. Recent studies have revealed that GBV-C/HGV RNA is cleared spontaneously with a time overlap of GBV-C/HGV viremia and anti-E2 seroconversion after chronic infection in a majority of patients [Chen et al., 1997; Enomoto et al., 1998; Prati et al., 1998]. Because our subjects were younger than the subjects in other studies, it is possible that none of the patients in our study was able to clear GBV-C/HGV viremia. Accordingly, there would be a strong possibility of spontaneous recovery in the future among young patients, especially those patients positive for both markers. To determine whether the age of patients influences the clearance of GBV-C/HGV from the circulation, further long-term longitudinal studies of GBV-C/HGV RNA-positive and anti-E2-negative young patients are needed. Second, immunosuppressive therapy for underlying malignant disease might prevent the host's immune system from eliminating GBV-C/HGV spontaneously. In fact, we identified underlying malignant disease as a risk factor for GBV-C/HGV viremia. This finding is compatible with previous studies indicating that immunosuppression might increase the risk of GBV-C/HGV infection [Kudo et al., 1996; Neilson et al., 1996].

Previously, a few studies had reported that GBV-C/HGV was detected in 3.5–8% children with chronic HCV infection [Bortolotti et al., 1997; Lopez-Alcorocho et al., 1997]. The difference may be attributable to the source of GBV-C/HGV infection. Blood transfusion is considered to be a main route of GBV-C/HGV transmis-

sion. Fifty-three (82.8%) of our patients had a history of blood transfusion, whereas the proportion was 19 (33.3%) of the 57 patients in the study by Bortolotti et al. [1997]. In addition, immunosuppressive treatment might have increased the prevalence of GBV-C/HGV infection in our study.

Several studies have been conducted to investigate how GBV-C/HGV affects the clinical characteristics of adult patients with chronic hepatitis C; however, GBV-C/HGV did not seem to have any effect on the severity of chronic hepatitis C [Enomoto et al., 1998; Oshita et al., 1998; Petrik et al., 1998; Shev et al., 1998]. In our study, there were no specific clinical, virological, or histological characteristics noted distinguishing GBV-C/HGV RNA-positive patients from GBV-C/HGV RNA-negative patients, except for underlying malignant disease. These findings suggest that GBV-C/HGV may not play a significant pathogenic role in young patients with chronic hepatitis C having a history of malignant disease.

IFN therapy is more effective in children with chronic hepatitis C than in adult patients [Fujisawa et al., 1995; Komatsu et al., 1996]. Although the number of patients examined was small, this study showed that GBV-C/HGV infection did not modify the response to IFN therapy in patients with chronic hepatitis C. All of the six GBV-C/HGV RNA-positive patients treated with IFN became GBV-C/HGV RNA-negative 3 months after the start of IFN therapy, but five of them became positive for GBV-C/HGV RNA at the time of or after completion of IFN therapy (Fig. 1). These findings suggest that IFN therapy can suppress the replication of GBV-C/HGV transiently, but it is not effective in cases of persistent GBV-C/HGV infection.

In conclusion, GBV-C/HGV infection was not uncommon among young patients with chronic hepatitis C, but anti-E2 was detectable infrequently. Underlying malignant disease was found to be a risk factor for GBV-C/HGV viremia. Further studies are required to determine whether spontaneous recovery from GBV-C/HGV infection is associated with age. The data provide no evidence to suggest that GBV-C/HGV might affect the clinical course of young patients with chronic HCV infection.

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